**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dissolve in mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Column:** 10 μm Spherisorb ODS
**Mobile phase:** MeCN:water:acetic acid 35:64:1
**Injection volume:** 100
**Detector:** UV 240

**CHROMATOGRAM**
**Internal standard:** fluocinonide

**KEY WORDS**
nasal spray

**REFERENCE**
Yu,C.D.; Jones,R.E.; Henesian,M. Cascade impactor method for the droplet size characterization of a metered-dose nasal spray, *J.Pharm.Sci.*, **1984**, *73*, 344–348.

**SAMPLE**
**Matrix:** solutions

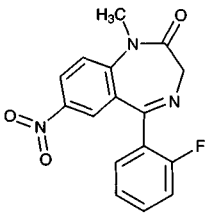
**HPLC VARIABLES**
**Column:** 300 × 3.9 μBondapak C18
**Mobile phase:** MeOH:water 55:45
**Flow rate:** 2
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 10.2

**OTHER SUBSTANCES**
**Simultaneous:** metabolites

**REFERENCE**
Tökés,L.; Cho,D.; Maddox,M.L.; Chaplin,M.D.; Chu,N.I. Isolation and identification of an oxidatively defluorinated metabolite of flunisolide in man, *Drug Metab.Dispos.*, **1981**, *9*, 485–486.

# Flunitrazepam



**Molecular formula:** $C_{16}H_{12}FN_3O_3$
**Molecular weight:** 313.29
**CAS Registry No.:** 1622-62-4
**Merck Index:** 4181
**Lednicer No.:** 2 406

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Vortex 1 mL plasma with 3 mL toluene:isoamyl alcohol 95:5 at 1000 rpm for 90 s, centrifuge at 2600 g for 10 min. Evaporate a 2.5 mL aliquot of the upper organic layer to dryness under nitrogen at 40°, reconstitute with 100 μL mobile phase, inject a 25 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 10 × 4.6 5 μm Nucleosil 120-5 C18

**Column:** 250 × 4 5 μm Nucleosil 120-5 C 18
**Mobile phase:** MeOH:buffer 47:53 (Buffer was 100 mM $Na_2HPO_4$ adjusted to pH 7.8 with orthophosphoric acid.)
**Column temperature:** 37
**Flow rate:** 1.2
**Injection volume:** 25
**Detector:** UV 302

**CHROMATOGRAM**
**Retention time:** 11.4
**Internal standard:** flunitrazepam

**OTHER SUBSTANCES**
**Extracted:** omeprazole

**KEY WORDS**
plasma; flunitrazepam is IS

**REFERENCE**
Macek,J.; Ptácek,P.; Klíma,J. Determination of omeprazole in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *689*, 239–243.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 4 μm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 221

**CHROMATOGRAM**
**Retention time:** 3.92
**Limit of detection:** <120 ng/mL

**KEY WORDS**
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Condition a 300 mg Bond Elut Certify LRC SPE cartridge with 2 volumes of the elution mixture, MeOH, and pH 6 phosphate buffer. Add 30 μL 10 μg/mL IS in MeOH and 5 mL pH 6 phosphate buffer to 1 mL serum, plasma or urine. Vortex, centrifuge at 10000 rpm for 5 min. Add the supernatant to the SPE cartridge and allow to pass through at 1.0 mL/min. Wash with 5 mL MeOH:water 20:80, 5 mL 1 M orthophosphoric acid, 5 mL MeOH, and 5 mL chloroform. Elute with 2 mL chloroform:isopropanol:ammonia 70:28:2. Add 20 μL ethylene glycol to the effluent, evaporate at 50° under vacuum. Add 30 μL mobile phase to the residue. Inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen! Prepare the SPE elution mixture as follows. Add 400 μL ammonia to 19.6 mL chloroform:isopropanol 70:30.)

## HPLC VARIABLES

**Column:** 250 × 4.0 LiChrospher 60 RP-Select B
**Mobile phase:** MeCN:20 mM pH 2.0 phosphate buffer 36:64
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 16.19
**Internal standard:** methylclonazepam (19.97)
**Limit of detection:** 1 ng/mL

## OTHER SUBSTANCES

**Extracted:** metabolites

**Simultaneous:** alprazolam, bromazepam, brotizolam, clobazam, clonazepam, desmethyldiazepam, diazepam, lorazepam, midazolam, methylclonazepam, nitrazepam, oxazepam, temazepam, triazolam

**Noninterfering:** amphetamine, atropine, barbital, caffeine, cocaine, codeine, dronabinol, fluphenazine, haloperidol, imipramine, morphine, phenobarbital, secobarbital

## KEY WORDS

serum; plasma; SPE

## REFERENCE

Deinl,I.; Mahr,G.; von Meyer,L. Determination of flunitrazepam and its main metabolites in serum and urine by HPLC after mixed-mode solid-phase extraction, *J.Anal.Toxicol.*, **1998**, *22*, 197–202.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 200.5

## CHROMATOGRAM

**Retention time:** 18.558

## KEY WORDS

whole blood

## REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

## OTHER SUBSTANCES

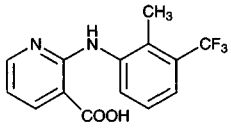
**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fen-

camfamine, fenoprofen, fenproporex, fentanyl, flubendazole, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

**REFERENCE**

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

# Flunixin




**Molecular formula:** $C_{14}H_{11}F_3N_2O_2$
**Molecular weight:** 296.25
**CAS Registry No.:** 38677-85-9, 42461-84-7 (meglumine salt)
**Merck Index:** 4182
**Lednicer No.:** 2 281

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1 mL Plasma or serum + 4 mL 250 ng/mL naproxen in MeCN, vortex for 30 s, centrifuge at 1000 g for 15 min. Remove 4 mL of the supernatant and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μL mobile phase, inject a 50 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 50 mm long 30 μm pellicular ODS
**Column:** 250 mm long 5 μm Spherisorb ODS I
**Mobile phase:** MeCN:MeOH:1% pH 3.0 acetate buffer 30:20:50
**Flow rate:** 1.2
**Injection volume:** 50
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 9.0

**Internal standard:** naproxen (7.7)
**Limit of detection:** 50 ng/mL

### OTHER SUBSTANCES
**Extracted:** phenylbutazone, oxyphenbutazone

### KEY WORDS
plasma; serum; horse; pharmacokinetics; for dogs see Am.J.Vet.Res. 1985; 46; 235

### REFERENCE
Hardee,G.E.; Lai,J.-W.; Moore,J.N. Simultaneous determination of flunixin, phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone in equine plasma by high-performance liquid chromatography: With application to pharmacokinetics, *J.Liq.Chromatogr.*, **1982**, *5*, 1991–2003.

### SAMPLE
**Matrix:** blood, milk
**Sample preparation:** Plasma. 1 mL Plasma + 10 μL 6 μg/mL IS + 1 mL 1 M HCl + 3 mL water, add to a Clin Elut SPE cartridge, elute with two 8 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL MeOH, vortex for 30 s, inject a 50 μL aliquot. Milk. 25 mL Milk + 2.5 mL 1 M HCl, mix briefly, centrifuge at 10000 rpm in a refrigerated centrifuge for 10 min. Remove the supernatant and wash the pellet with 15 mL 1 M HCl, centrifuge at 10000 rpm in a refrigerated centrifuge for 10 min. Combine the supernatants, add a 5 mL aliquot to 150 μL 10 μg/mL IS and 5 mL hexane, rotate for 5 min, centrifuge at 1000 g for 5 min, add the aqueous phase to a Clin Elut SPE cartridge, elute with two 8 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL MeOH, vortex for 30 s, inject a 50 μL aliquot.

### HPLC VARIABLES
**Guard column:** 40 μm CO:PELL ODS
**Column:** Radial Compression C18 (Waters)
**Mobile phase:** MeCN:25 mM pH 2.5 potassium phosphate buffer 50:50
**Flow rate:** 2
**Injection volume:** 50
**Detector:** UV 280

### CHROMATOGRAM
**Retention time:** 3.2
**Internal standard:** Sch 13476 (4.8)
**Limit of quantitation:** 50 ng/mL

### KEY WORDS
plasma; cow; SPE

### REFERENCE
Neff-Davis,C.A.; Bosch,K. An HPLC method for the determination of flunixin in bovine plasma and milk, *J.Vet.Pharmacol.Ther.*, **1985**, *8*, 331–334.

### SAMPLE
**Matrix:** blood, urine
**Sample preparation:** 5 mL Blood or 10 mL urine + 2.5 (blood) or 5 (urine) μg flufenamic acid, acidify with 2 M pH 4.5 acetate buffer, add dichloromethane:EtOH 95:5, agitate for 3 min, centrifuge at 2000 g for 10 min, repeat extraction. Combine the organic phases, wash with saturated sodium bicarbonate solution, evaporate to dryness under a stream of air at 45°, reconstitute in MeOH, inject an aliquot.

### HPLC VARIABLES
**Guard column:** 4 × 4 5 μm Lichrospher 100 RP-18
**Column:** 125 × 4 5 μm Lichrospher 100 RP-18
**Mobile phase:** MeOH:buffer 70:30 (Buffer was 200 mM $Na_2HPO_4$ and 100 mM citric acid, pH 3.2.)

**Flow rate:** 1
**Detector:** UV 284

**CHROMATOGRAM**
**Internal standard:** flufenamic acid
**Limit of quantitation:** 100 ng/mL (urine), 250 ng/mL (blood)

**KEY WORDS**
horse; pharmacokinetics

**REFERENCE**
Araújo,A.C.; Salvadori,M.C.; Velletri,M.E.; Camargo,M.M.A. Influence of furosemide on the detection of flunixin meglumine in horse urine samples, *J.Anal.Toxicol.*, **1990**, *14*, 146–148.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Plasma. 1 mL Plasma + 50 μL 500 ng/mL mefenamic acid or indomethacin + 1 mL 100 mM HCl + 10 mL dichloromethane, rotate for 10 min, centrifuge at 1500 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Redissolve the residue in mobile phase, inject a 20 μL aliquot. Urine. 50 μL Urine + 1 mL mobile phase, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 75 × 4.6 3 μm Supelcosil LC-8
**Mobile phase:** MeCN:50 mM phosphoric acid 45:55
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 235

**CHROMATOGRAM**
**Retention time:** 2.8
**Internal standard:** mefenamic acid (8) or indomethacin (5)
**Limit of detection:** 50-250 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** naproxen, thiosalicylic acid,ethacrynic acid, phenylbutazone

**KEY WORDS**
plasma

**REFERENCE**
Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *568*, 351–361.

**SAMPLE**
**Matrix:** milk
**Sample preparation:** Condition a 3 mL C18 Bakerbond SPE cartridge with 5 mL ethyl acetate and 5 mL water. 5 mL Milk + 200 μL 4000 U/mL β-glucuronidase (type IX-A, Sigma) in 100 mM pH 6.8 phosphate buffer, heat at 37° for 2 h, cool, acidify to pH 3.0-3.5 with about 350-450 μL 1 M HCl, while vortexing gently, add 5.4-5.6 g 60-200 mesh silica gel (Baker No. 3405-1), mix thoroughly for 2 min, place in a 300 × 25 glass column on top off 0.5-1 g untreated silica gel, place 0.5-1 g untreated silica gel on the top of the column, wash with 50 mL water-saturated dichloromethane:hexane 30:70, elute with 50 mL ethyl acetate. Wash the eluate with 25 mL pH 3.5 HCl for 15 s, extract ethyl acetate layer twice with NaOH solution by shaking for 15 s. Combine the aqueous extracts and adjust the pH to 5.0-5.5 with 1 M HCl, add to the SPE cartridge, elute with 5 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 50-55°, reconstitute the residue in 500 μL MeOH:buffer 50:50, sonicate briefly, filter (0.45 μm), inject a 100 μL aliquot of the filtrate. (NaOH solution was 15 mL 100 mM

NaOH and 1 mL 1 M NaOH. Buffer was 2.5 mL 1 M tetrabutylammonium dihydrogen phosphate and 10 mL 100 mM NaOH made up to 500 mL with water, pH 6.)

**HPLC VARIABLES**
**Guard column:** 20 × 4 5 μm Hypersil C18 ODS
**Column:** 200 × 4.6 5 μm Hypersil C18 ODS
**Mobile phase:** MeOH:buffer 58:42 (Buffer was 2.5 mL 1 M tetrabutylammonium dihydrogen phosphate and 10 mL 100 mM NaOH made up to 500 mL with water, pH 6.)
**Column temperature:** 45
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 285

**CHROMATOGRAM**
**Retention time:** 5.7
**Limit of quantitation:** 1.7 ng/mL

**KEY WORDS**
cow; SPE

**REFERENCE**
Rupp,H.S.; Holland,D; Munns,R.K.; Turnipseed,S.B.; Long,A.R. Determination of flunixin in milk by liquid chromatography with confirmation by gas-chromatography/mass spectrometry and selected ion monitoring, *J.AOAC Int.*, **1995**, *78*, 959–967.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** Condition a 3 mL Bond Elut Certify II (C8 plus strong anion exchanger) SPE cartridge with 3 mL MeOH and 3 mL water. Adjust pH of urine to 7 with 1 M NaOH or 1 M HCl, centrifuge at 500 g for 15 min. Add 3 mL of the supernatant to the SPE cartridge at 0.2 mL/min, wash with two 2.5 mL portions of water, wash with two 2 mL portions of MeOH, dry for 20 min under full vacuum, wash with two 2 mL portions of hexane, elute with two 2 mL portions of hexane:acetic acid 90:10, add 2 μg ketoprofen. Evaporate to dryness under a stream of nitrogen, reconstitute in 100 μL hexane:isopropanol 50:50, inject an aliquot.

**HPLC VARIABLES**
**Column:** 100 × 2 5 μm Hypersil SI
**Mobile phase:** Gradient. Hexane:isopropanol containing 5% water from 98:2 to 70:30 over 8 min, to 0:100 over 4 min, maintain at 0:100 for 1 min, re-equilibrate at initial conditions for 5 min.
**Column temperature:** 45
**Flow rate:** 0.4
**Injection volume:** 3
**Detector:** UV 280 or MS, Hewlett-Packard HP5989A quadrupole, HP 59980B PB interface at 65° with helium pressure of 275 kPa, 10 kV high energy dynode, source 275°, particle beam, EI, CI (methane)

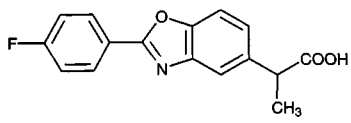
**CHROMATOGRAM**
**Retention time:** 4.53
**Internal standard:** ketoprofen (3.84)
**Limit of detection:** 10 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites
**Simultaneous:** alclofenac, aminoantipyrine, bumadizone, clonixin, diclofenac, diflunisal, dipyrone, famprofazone, fenbufen, fenclofenac, fenoprofen, floctafenine, flufenamic acid, flurbiprofen, ibufenac, ibuprofen, indomethacin, indoprofen, isopyrin, isoxepac, ketorolac, meclofenamic acid, naproxen, nefopam, niflumic acid, oxaprozin, phenazone, phenazopyridine, phenylbutazone, piroxicam, propyphenazone, salicylamide, sulindac, suprofen, tenoxicam, tiaprofenic acid, tolmetin, zomepirac

**KEY WORDS**
horse; SPE; normal phase

**REFERENCE**
Stanley,S.M.; Owens,N.A.; Rodgers,J.P. Detection of flunixin in equine urine using high-performance liquid chromatography with particle beam and atmospheric pressure ionization mass spectrometry after solid-phase extraction, *J.Chromatogr.B*, **1995**, *667*, 95–103.

# Flunoxaprofen



**Molecular formula:** $C_{16}H_{12}FNO_3$
**Molecular weight:** 285.27
**CAS Registry No.:** 66934-18-7
**Merck Index:** 4183

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** 50 μL Plasma or urine + 10 μL 1 M NaOH, heat at 37° for 2 h, add 10 μL 1 M HCl, extract with 1 mL ethyl acetate. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL 50 mM triethylamine in MeCN, add 50 μL 60 mM ethyl chloroformate in MeCN, let stand for 2 min, add 50 μL 1 M L-leucinamide in 1 M triethylamine in MeOH. Evaporate, take up the residue in 100 μL mobile phase, inject a 100 μL aliquot. (Hydrolysis of glucuronides may be omitted.)

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Ultrasphere ODS
**Mobile phase:** MeCN:60 mM pH 6 potassium phosphate buffer 40:60
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 272

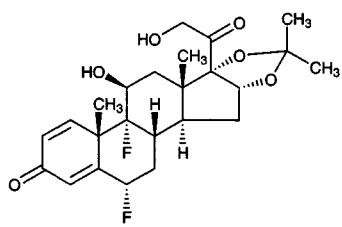
**CHROMATOGRAM**
**Retention time:** 21.2 (R), 23.8 (S)
**Internal standard:** flunoxaprofen

**OTHER SUBSTANCES**
**Extracted:** ketoprofen, fenoprofen

**KEY WORDS**
plasma; chiral; flunoxaprofen is IS; derivatization

**REFERENCE**
Volland,C.; Sun,H.; Benet,L.Z. Stereoselective analysis of fenoprofen and its metabolites, *J.Chromatogr.*, **1990**, *534*, 127–138.

# Fluocinolone acetonide



**Molecular formula:** $C_{24}H_{30}F_2O_6$
**Molecular weight:** 452.20
**CAS Registry No.:** 67-73-2
**Merck Index:** 4185
**Lednicer No.:** 1 202; 3 94

**SAMPLE**
**Matrix:** blood

**Sample preparation:** 100 μL Plasma + 1.2 mL dichloromethane, shake 1 min, repeat extraction. Combine organic layers and evaporate a 2 mL aliquot under reduced pressure below 40°. Dissolve the residue in 100 μL MeCN, add 10 μL reagent 1, add 10 μL reagent 2, heat at 70° for 20 min, cool to room temperature, add 100 μL water, add 200 μL MeOH:water 1:1, apply to a Sep-Pak C18 cartridge, wash vial into cartridge with 2 mL MeOH:water 1:1, wash cartridge with 40 mL MeOH:water 1:1, elute with 5 mL MeOH. Concentrate eluate to 500 μL by evaporation under reduced pressure below 40°, inject 20 μL aliquot. (Reagent 1 was 30 mg 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole in 3 mL pyridine, add 700 mg 4-piperidinopyridine, dilute to 10 mL with MeCN. Reagent 2 was 700 mg 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate in 10 mL MeCN. Prepare 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as follows. Add 13 g 4-carboxybenzaldehyde (terephthalaldehydic acid) in 400 mL EtOH dropwise to 4,5-dimethyl-1,2-phenylenediamine in 400 mL EtOH in an ice bath, after 1 h reflux for 8 h, cool to room temperature, collect the precipitate, recrystallize three times from MeOH:water 50:50 to give 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as a white amorphous product (mp >300°) (J.Chromatogr. 1991, 585, 219). 4-Piperidinopyridine is not commercially available but 4-dimethylaminopyridine or 4-pyrrolidinopyridine can be used instead although interferences are greater (J. Chromatogr. 1991, 585, 219). Alternatively 4-piperidinopyridine can be synthesized as follows. Add 200 mmoles piperidine dropwise with stirring to 15 g phosphorus pentoxide and 9.51 g 4-hydroxypyridine, heat at 250° for 7 h, cautiously pour onto 200 g ice, add 400 mL 1 M NaOH, add 200 mL ether. Remove the ether layer and extract the aqueous layer three times with 100 mL portions of ether. Combine the organic layers and dry them over anhydrous potassium carbonate, evaporate, distil the residue, recrystallize from petroleum ether (bp 80-100°) to give 4-piperidinopyridine (bp 167-170°/11 mm Hg; mp 79-80°) (Synthesis 1978, 844). Alternatively, add 1.94 g 4-bromopyridine hydrochloride to 5 mL 50% NaOH, add 5 mL piperidine, add 2.72 g benzyltriethylammonium bromide, heat at 100° for 5 h, remove excess piperidine by distillation, add 25 mL water, extract four times with 25 mL portions of benzene. Combine the organic layers and dry them over anhydrous sodium sulfate, boil the residue with petroleum ether to give 4-piperidinopyridine (mp 80°) (Syn. Commun. 1979, 9, 251). Prepare 1-isopropyl-3-(3-dimethylaminopropyl) carbodiimide perchlorate as follows. Stir 1.41 moles isopropylisocyanate in 750 mL dichloromethane at 5°, add 144 g 3-dimethylaminopropylamine (N,N-dimethyl-1,3-propanediamine) in 250 mL dichloromethane at such a rate that the temperature does not exceed 10°, add 500 mL triethylamine, add 300 g p-toluenesulfonyl chloride in 300 mL dichloromethane at such a rate that the temperature does not exceed 10°, reflux for 3 h, add 400 g anhydrous sodium carbonate, add 3.5 L ice water, stir vigorously for 30 min, remove the organic phase. Extract the aqueous phase three times with 500 mL portions of dichloromethane. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate under reduced pressure, distil the residue to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide (bp 91-92°/10 mm Hg (Ber. 1941, 74B, 1285)) (cf. Org. Syn. 1973, Coll. Vol. V, 555). Prepare pyridine perchlorate from pyridine and 20% perchloric acid, crystallize from EtOH (Ber. 1926, 59, 446). Add 18 g pyridine perchlorate in portions to 100 mmoles 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide stirred in 200 mL dichloromethane at 0°, let stand for 30 min, filter, add 200 mL anhydrous diethyl ether to the filtrate. Filter off the precipitate and recrystallize it from dichloromethane/diethyl ether to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate (mp 88-90°) (Chem. Pharm. Bull. 1985, 33, 5375).)

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**HPLC VARIABLES**
**Guard column:** 50 × 4.6 7 μm Zorbax ODS
**Column:** 250 × 4.6 7 μm Zorbax ODS
**Mobile phase:** MeOH:water 75:25 containing 5 mM tetramethylammonium hydrogen sulfate
**Flow rate:** 0.4
**Injection volume:** 20
**Detector:** F ex 334 em 418

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**CHROMATOGRAM**
**Retention time:** 40.7
**Internal standard:** fluocinolone acetonide
**Limit of detection:** 0.6 pg/mL

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**OTHER SUBSTANCES**
**Extracted:** triamcinolone, triamcinolone acetonide
**Simultaneous:** aldosterone, cortisone, hydrocortisone, corticosterone, fluocinolone acetonide, triamcinolone, triamcinolone acetonide, dexamethasone

**KEY WORDS**
plasma; derivatization; fluocinolone acetonide is IS

**REFERENCE**
Katayama,M.; Masuda,Y.; Taniguchi,H. Determination of corticosteroids in plasma by high-performance liquid chromatography after pre-column derivatization with 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole, *J.Chromatogr.*, **1993**, *612*, 33–39.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 3.9 4 μm Nova-Pak C18
**Mobile phase:** Gradient. A was 58 mM $NaH_2PO_4$ containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 245, 256, 343

**CHROMATOGRAM**
**Retention time:** 24.75 (fluocinolone acetonide)
**Internal standard:** 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
**Limit of detection:** 1-10 ng/mL (245 nm)

**OTHER SUBSTANCES**
**Extracted:** betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone
**Noninterfering:** aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

**KEY WORDS**
serum

**REFERENCE**
Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids, *J.Chromatogr.B*, **1995**, *666*, 347–353.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dissolve 5 g cream containing 0.00925% fluocinonide, 0.00365% procinonide, and 0.0021% ciprocinonide in 2.5 mL THF, add norethindrone, dilute to 25 mL with MeOH, centrifuge, inject a 25 μL aliquot onto column A with mobile phase A and allow components to elute from column A to column B for 7 min. After 7 min remove column A from circuit, monitor effluent from column B. Back-flush column A with mobile phase B for 5 min, equilibrate column A with mobile phase A for 5 min before next injection.

**HPLC VARIABLES**
**Column:** A 30 × 4.6 5 μm Spheri-5 ODS (Brownlee); B 70 × 2.1 Whatman Co:Pell ODS + 250 × 4.6 5 μm Ultrasphere C18
**Mobile phase:** A MeCN:THF:water 43:4:53; B MeOH:THF 75:25
**Flow rate:** A 1.5; B 1
**Injection volume:** 25

**Detector:** UV 260 for 22 min then UV 236

**CHROMATOGRAM**
**Retention time:** 8 (fluocinolone acetonide)
**Internal standard:** norethindrone (12)

**OTHER SUBSTANCES**
**Simultaneous:** procinonide, ciprocinonide, fluocinonide

**KEY WORDS**
creams; column-switching

**REFERENCE**
Conley,D.L.; Benjamin,E.J. Automated high-performance liquid chromatographic column switching technique for the on-line clean-up and analysis of drugs in topical cream formulations, *J.Chromatogr.*, **1983**, *257*, 337–344.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** 11.25 g Cream + 100 mL cyclohexane + 50 mL MeOH, shake vigorously for 3 min, let stand for 15 min. Remove the lower layer and add it to 140 mL water and 100 mL chloroform, shake for 3 min, allow phases to separate. Remove a 3 mL aliquot of the chloroform layer and add it to 300 μL 0.028% hydrocortisone in EtOH, add 1 mL 3 mg/mL acenaphthene-5-sulfonyl hydrazine in EtOH:toluene 10:90, evaporate to dryness under reduced pressure at 60°, reconstitute with 200 μL mobile phase, inject an aliquot. (Preparation of acenaphthene-5-sulfonyl hydrazine is as follows. Dissolve 20 g acenaphthene in 100 g nitrobenzene, cool to 0°, add 9 mL chlorosulfonic acid dropwise with stirring, maintain the temperature below 5°, when the addition is complete allow the temperature to rise to 20° over 30 min, add 500 mL water. Remove the aqueous layer and neutralize it with solid sodium carbonate, heat and add NaCl until precipitation occurs, cool in an ice bath for 1 h, filter, heat at 140° to remove traces of water and nitrobenzene to give acenaphthene-5-sulfonic acid sodium salt as a pale yellow solid (mp >300°). Grind 10 g acenaphthene-5-sulfonic acid sodium salt with 3.5 g phosphorus pentachloride in a mortar for 3 min, add ice and water, extract with 100 mL ethyl acetate. Wash the ethyl acetate layer with 5% sodium bicarbonate and with water until neutral, dry over anhydrous sodium sulfate, evaporate the ethyl acetate under a stream of nitrogen, chromatograph on a 300 × 20 column of silica gel H with toluene to give acenaphthene-5-sulfonyl chloride (mp 98-101°) as the first yellow band to elute. Cool a solution of 1 g acenaphthene-5-sulfonyl chloride in 3 mL THF to 10° and pass nitrogen through the solution, add 400 μL 85% hydrazine hydrate dropwise with stirring, maintain the temperature between 10° and 15°, stir for a further 15 min. Filter the upper THF layer through Celite, wash the Celite with 1 mL THF. Stir the filtrate vigorously and add two 10 mL portions of water, cool in a refrigerator for 1 h, filter the precipitate, wash with water, dry, recrystallize from EtOH to give acenaphthene-5-sulfonyl hydrazine (mp 132-4°).)

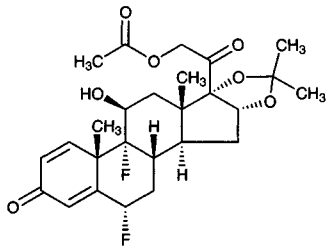
**HPLC VARIABLES**
**Column:** 500 × 1 10 μm silica
**Mobile phase:** Toluene:dioxane 90:10 (Caution! Dioxane is a carcinogen!)
**Detector:** F ex 230 em 350

**CHROMATOGRAM**
**Internal standard:** hydrocortisone

**KEY WORDS**
derivatization; cream; normal phase; for fluocinolone acetonide

**REFERENCE**
Gifford,L.A.; Owusu-Daaku,F.T.K.; Stevens,A.J. Acenaphthene fluorescence derivatization reagents for use in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *715*, 201–212.

# Fluocinonide



**Molecular formula:** $C_{26}H_{32}F_2O_7$
**Molecular weight:** 494.53
**CAS Registry No.:** 356-12-7
**Merck Index:** 4186

## SAMPLE

**Matrix:** formulations
**Sample preparation:** Dissolve 5 g cream containing 0.00925% fluocinonide, 0.00365% procinonide, and 0.0021% ciprocinonide in 2.5 mL THF, add norethindrone, dilute to 25 mL with MeOH, centrifuge, inject a 25 μL aliquot onto column A with mobile phase A and allow components to elute from column A to column B for 7 min. After 7 min remove column A from circuit, monitor effluent from column B. Back-flush column A with mobile phase B for 5 min, equilibrate column A with mobile phase A for 5 min before next injection.

## HPLC VARIABLES

**Column:** A 30 × 4.6 5 μm Spheri-5 ODS (Brownlee); B 70 × 2.1 Whatman Co:Pell ODS + 250 × 4.6 5 μm Ultrasphere C18
**Mobile phase:** A MeCN:THF:water 43:4:53; B MeOH:THF 75:25
**Flow rate:** A 1.5; B 1
**Injection volume:** 25
**Detector:** UV 260 for 22 min then UV 236

## CHROMATOGRAM

**Retention time:** 18
**Internal standard:** norethindrone (12)

## OTHER SUBSTANCES

**Simultaneous:** procinonide, ciprocinonide, fluocinolone acetonide

## KEY WORDS

creams; column-switching

## REFERENCE

Conley,D.L.; Benjamin,E.J. Automated high-performance liquid chromatographic column switching technique for the on-line clean-up and analysis of drugs in topical cream formulations, *J.Chromatogr.*, **1983**, *257*, 337–344.

## SAMPLE

**Matrix:** formulations
**Sample preparation:** Dissolve in mobile phase, inject an aliquot.

## HPLC VARIABLES

**Column:** 10 μm Spherisorb ODS
**Mobile phase:** MeCN:water:acetic acid 35:64:1
**Injection volume:** 100
**Detector:** UV 240

## CHROMATOGRAM

**Internal standard:** fluocinonide

## OTHER SUBSTANCES

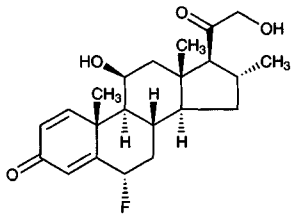
**Simultaneous:** flunisolide

## KEY WORDS

nasal spray; fluocinonide is IS

**REFERENCE**
Yu,C.D.; Jones,R.E.; Henesian,M. Cascade impactor method for the droplet size characterization of a metered-dose nasal spray, *J.Pharm.Sci.*, **1984**, *73*, 344–348.

# Fluocortolone



**Molecular formula:** $C_{22}H_{29}FO_4$
**Molecular weight:** 376.47
**CAS Registry No.:** 152-97-6, 303-40-2 (21-hexanoate)
**Merck Index:** 4188

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Add 150 µL MeOH to 1 mL plasma. Add 500 µL 100 mM NaOH and 2 mL dichloromethane, shake for 10 min, centrifuge at 2500 g for 10 min, evaporate a 1.9 mL aliquot of the supernatant under a stream of nitrogen at 45°. Reconstitute the residue in 50 µL MeOH, inject 17 µL aliquot.

**HPLC VARIABLES**
**Guard column:** 10 × 4 5 µm LiChrospher RP 18
**Column:** 250 × 4 5 µm Lichrospher RP 18
**Mobile phase:** MeOH:THF:water 110:2.5:100
**Flow rate:** 1
**Injection volume:** 17
**Detector:** UV 252

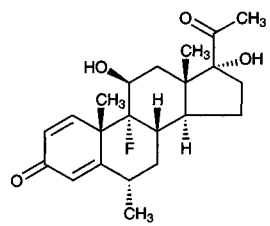
**CHROMATOGRAM**
**Internal standard:** fluocortolone

**OTHER SUBSTANCES**
**Extracted:** hydrocortisone, triamcinolone

**KEY WORDS**
plasma

**REFERENCE**
Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 79–88.

# Fluorometholone



**Molecular formula:** $C_{22}H_{29}FO_4$
**Molecular weight:** 376.47
**CAS Registry No.:** 426-13-1, 3801-06-7 (acetate)
**Merck Index:** 4213
**Lednicer No.:** 1 203

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Plasma + 5 mL hexane, shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min, discard the hexane layer, add 8 mL dichloromethane,

shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min, repeat extraction with 8 mL dichloromethane. Combine the dichloromethane layers and add 300 mg anhydrous sodium sulfate, shake horizontally at 200 cycles/min for 5 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, add 2 mL hexane, vortex for 1 min, centrifuge at 1000 g for 5 min, discard the hexane layer, inject a 100 μL aliquot of the mobile phase layer.

**HPLC VARIABLES**
**Guard column:** 30 × 4.6 5 μm Spheri-5 RP-18
**Column:** 250 × 4.6 5 μm Ultrasphere ODS
**Mobile phase:** MeCN:water:glacial acetic acid 33:62:5
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 14.55
**Internal standard:** fluorometholone

**OTHER SUBSTANCES**
**Extracted:** methylprednisolone, methylprednisolone acetate

**KEY WORDS**
plasma; fluorometholone is IS

**REFERENCE**
Hopkins,N.K.; Wagner,C.M.; Brisson,J.; Addison,T.E. Validation of the simultaneous determination of methylprednisolone and methylprednisolone acetate in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *577*, 87–93.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Plasma + 5 mL hexane, shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min. Remove the aqueous layer and add it to 8 mL dichloromethane, shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and add them to 300 mg anhydrous sodium sulfate, shake horizontally at 200 cycles/min for 5 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, add 2 mL hexane, vortex for 1 min, centrifuge at 1000 g for 5 min, inject a 100 μL aliquot of the aqueous layer.

**HPLC VARIABLES**
**Guard column:** 30 × 4.6 5 μm Brownlee guard column
**Column:** 250 × 4.6 5 μm Ultrasphere ODS
**Mobile phase:** MeCN:water:glacial acetic acid 33:62:5
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 14.6
**Internal standard:** fluorometholone

**OTHER SUBSTANCES**
**Extracted:** methylprednisolone, methylprednisolone acetate

**KEY WORDS**
plasma; fluorometholone is IS

### REFERENCE

Hopkins,N.K.; Wagner,C.M.; Brisson,J.; Addison,T.E. Validation of the simultaneous determination of methylprednisolone and methylprednisolone acetate in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *577*, 87–93.

### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

### HPLC VARIABLES

**Column:** 150 × 3.9 4 μm Nova-Pak C18

**Mobile phase:** Gradient. A was 58 mM $NaH_2PO_4$ containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 245, 256, 343

### CHROMATOGRAM

**Retention time:** 26.15

**Internal standard:** 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)

**Limit of detection:** 1-10 ng/mL (245 nm)

### OTHER SUBSTANCES

**Extracted:** betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone

**Noninterfering:** aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

### KEY WORDS

serum

### REFERENCE

Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids, *J.Chromatogr.B*, **1995**, *666*, 347–353.

### SAMPLE

**Matrix:** solutions

**Sample preparation:** Dilute in an appropriate solvent, inject an aliquot.

### HPLC VARIABLES

**Guard column:** RC18 Guardpak (Waters)

**Column:** 250 × 4.5 μBondapak C18

**Mobile phase:** MeCN:water 40:60

**Flow rate:** 1.5

**Detector:** UV 246

### CHROMATOGRAM

**Retention time:** 6.8

### OTHER SUBSTANCES

**Simultaneous:** 20-α-dihydrofluorometholone, prednisolone

**Interfering:** prednisolone acetate

### REFERENCE

Richman,J.B.; Tang-Liu,D.D.-S. A corneal perfusion device for estimating ocular bioavailability in vitro, *J.Pharm.Sci.*, **1990**, *79*, 153–157.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 150 × 4.5 5 μm Ultrasphere octyl
**Mobile phase:** MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35
**Column temperature:** 30
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 280

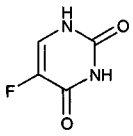
**CHROMATOGRAM**
**Retention time:** 3.19
**Internal standard:** naproxen (3.89)

**OTHER SUBSTANCES**
**Simultaneous:** bacitracin, cortisone acetate, diazepam, diclofenac, flurbiprofen, hydrocortisone acetate, imipramine, indomethacin, ketoprofen, ketorolac tromethamine, levobunolol, meclofenamic acid, metipranolol, neomycin, prednisolone acetate, proparacaine, propranolol, salicylic acid, sulfacetamide, suprofen
**Noninterfering:** acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide

**KEY WORDS**
human; rabbit

**REFERENCE**
Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

# Fluorouracil



**Molecular formula:** $C_4H_3FN_2O_2$
**Molecular weight:** 130.08
**CAS Registry No.:** 51-21-8
**Merck Index:** 4219
**Lednicer No.:** 3 155

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 150 μL plasma with 150 μL 10% trichloroacetic acid in water, vortex for 30 s, centrifuge at 5000 g for 5 min, inject a 50 μL aliquot of the clear supernatant.

**HPLC VARIABLES**
**Guard column:** 9 μm Aminex HPX-87H
**Column:** 300 × 7.8 9 μm Aminex HPX-87H
**Mobile phase:** 5 mM sulfuric acid
**Column temperature:** 60
**Flow rate:** 0.5
**Injection volume:** 50
**Detector:** UV 265

**CHROMATOGRAM**
**Retention time:** 24
**Limit of detection:** 25 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** cisplatin, 5-fluoro-2'-deoxyuridine-5'-monophosphate, 5-fluoro-2'-deoxyuridine, 5-fluorouridine, thymidine, uric acid, uridine
**Noninterfering:** alizapride, cytosine, hypoxanthine, methylprednisolone, methotrexate, metoclopramide, xanthine
**Interfering:** uracil

**KEY WORDS**
plasma; pharmacokinetics

**REFERENCE**
Compagnon,P.; Thiberville,L.; Moore,N.; Thuillez,C.; Lacroix,C. Simple high-performance liquid chromatographic method for the quantitation of 5-fluorouracil in human plasma, *J.Chromatogr.B*, **1996**, *677*, 380–383.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 500 μL plasma with 50 μL 5 μg/mL 5-chlorouracil, add to an unactivated C18 Chem Elut SPE cartridge (Varian). Elute with four 2 mL portions of MeOH:ethyl acetate 5:95. Flush the SPE cartridge with nitrogen at 1 mL/min. Evaporate collected eluate to dryness under a stream of nitrogen at 40° for 30 min. Reconstitute the residue in 200 μL water, sonicate for 10 min, filter (0.45 μm HV4 membrane, Millipore) Inject a 20 μL aliquot; SPE

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm Kromasil C18 (Touzart and Matignon, France)
**Mobile phase:** MeOH:water 3:97
**Flow rate:** 0.6
**Injection volume:** 20
**Detector:** UV 268

**CHROMATOGRAM**
**Retention time:** 5.8
**Internal standard:** 5-chlorouracil (10.7), 5-iodouracil (22.1)
**Limit of detection:** 10 ng/mL
**Limit of quantitation:** 20 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** metabolites (UV 275)

**KEY WORDS**
plasma; SPE

**REFERENCE**
Joulia,J.M.; Pinguet,F.; Grosse,P.Y.; Astre,C.; Bressolle,F. Determination of 5-fluorouracil and its main metabolites in plasma by high-performance liquid chromatography: Application to a pharmacokinetic study, *J.Chromatogr.B*, **1997**, *692*, 427–435.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 200 μL plasma with 50 μL 500 mM pH 8.0 phosphate buffer, 100 μL 500 ng/mL 5-chlorouracil in water, and 8 mL ethyl acetate. Shake for 30 min and centrifuge at 2200 g for 10 min. Remove the ethyl acetate layer and evaporate it. Reconstitute the residue in 500 μL mobile phase with sonication for 10 min. Inject a 150 μL aliquot.

**HPLC VARIABLES**
**Column:** 100 × 4.6 Develosil 60-3 (Nomura Chemical, Japan)
**Mobile phase:** n-Hexane:ethyl acetate:formic acid:water 50:0.5:0.5:0.3
**Flow rate:** 0.9 for 20 min, 0.4 for 27 min, 0.9 for 8 min
**Injection volume:** 150
**Detector:** UV 264

**CHROMATOGRAM**
**Internal standard:** 5-chlorouracil
**Limit of detection:** 3 ng/mL

**KEY WORDS**
plasma; rat

**REFERENCE**
Fuse,E.; Takai,K.; Okuno,K.; Kobayashi,S. Hepatic extraction ratio of 5-fluorouracil in rats. Dose dependence and effect of uracil and interleukin-2, *Biochem.Pharmacol.*, **1996**, *52*, 561–568.

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**SAMPLE**
**Matrix:** blood
**Sample preparation:** Add 50 μL 1 M pH 4.8 sodium acetate buffer to 1 mL plasma (to adjust pH to 6.0), add 250 μL 200 mg/mL saturated sodium sulfate solution, vortex, add 7 mL water-saturated ethyl acetate, vortex vigorously for 90 s, centrifuge for 5 min. Add 1 mL 50 mM pH 11 phosphate buffer to a 6 mL aliquot of the organic layer, vortex, centrifuge. Aspirate the organic layer to waste, flush the residual solvent from the aqueous portion with nitrogen for about 10 min, add 10 μL 1 M sulfuric acid (to adjust the pH to neutral), inject a 125 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm YMC ODS-AQ C18
**Mobile phase:** Gradient. A was MeOH:10 mM pH 5.5 potassium phosphate buffer 50:50. B was 10 mM pH 5.5 potassium phosphate buffer. A:B 0:100 for 5 min, from 0:100 to 50:50 in 1 min, maintain at 50:50 for 3 min, from 50:50 to 0:100 in 1 min, maintain at 0:100 for 10 min
**Column temperature:** 40
**Flow rate:** 1.2
**Detector:** UV 266

**CHROMATOGRAM**
**Retention time:** 6.8-7.3
**Limit of quantitation:** 25 ng/mL

**OTHER SUBSTANCES**
**Extracted:** uracil, 5-chlorouracil

**KEY WORDS**
plasma

**REFERENCE**
Coe,R.A.; Earl,R.A.; Johnson,E.T.C.; Lee,J.W. Determination of 5-fluorouracil in human plasma by a simple and sensitive reversed-phase HPLC method, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1733–1741.

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**SAMPLE**
**Matrix:** blood
**Sample preparation:** 500 μL Serum + 500 μL physiological saline + 100 μL 500 mM $NaH_2PO_4$ + 8 mL ethyl acetate, extract, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 500 μg/mL 4-bromomethyl-7-methoxycoumarin in acetone:MeCN 1:2 containing 100 μg/mL 18-crown-6 and 1 mg/mL potassium carbonate, reflux in the dark for 45 min, cool, add valeric acid, reflux for 5 min, dilute with acetone, inject an aliquot.

**HPLC VARIABLES**
**Column:** 200 × 4 5 μm Nucleosil 5 C18
**Mobile phase:** MeOH:water 70:30
**Flow rate:** 0.8
**Detector:** F ex 346 em 395

**CHROMATOGRAM**
**Retention time:** 7
**Internal standard:** valeric acid (8)

**Limit of detection:** 100 fmole

## OTHER SUBSTANCES
**Extracted:** tegafur

## KEY WORDS
derivatization; serum

## REFERENCE
Iwamoto,M.; Yoshida,S.; Hirose,S. Fluorescence determination of 5-fluorouracil and 1-(tetrahydro-2-furanyl)-5-fluorouracil in blood serum by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *310*, 151–157.

## SAMPLE
**Matrix:** blood

**Sample preparation:** 100 μL Serum + 5 μL 42 μM 5-chlorouracil in acetone, mix, let stand at room temperature for 3 min, add 500 μL 100 mM pH 3.5 potassium phosphate buffer, add 2 mL ethyl acetate, vortex for 2 min, centrifuge at 1000 g for 5 min. Remove a 1.4 mL aliquot of the organic layer and evaporate it to dryness under reduced pressure. Add 20 mg solid potassium bicarbonate:anhydrous sodium sulfate 1:7 to the residue, add 50 μL 1.3 mM 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone in acetone, add 50 μL 1.5 mM 18-crown-6 in acetone, heat at 50° for 20 min, cool, inject a 10 μL aliquot. (Silanize all glassware. Synthesize 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane: ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°) (J. Chromatogr. 1985, 346, 227). 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone is also available from Dojindo Molecular Technologies, Inc., 3 Bethesda Metro Center, Suite 700, Bethesda MD 20814; (301) 664-8448; www.dojindo.co.jp.)

## HPLC VARIABLES
**Column:** 100 × 8 10 μm Radial Pak C18 (Waters) (Wash with MeOH at 2 mL/min for 20 min at the end of each day.)

**Mobile phase:** Gradient. MeOH:water 35:65 for 15 min, 50:50 for 25 min (step gradient), re-equilibrate at initial conditions for 20 min.

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** F ex 370 em 455

**CHROMATOGRAM**
**Retention time:** 28.1
**Internal standard:** 5-chlorouracil (32.5)
**Limit of detection:** 12.5 ng/mL

**OTHER SUBSTANCES**
**Extracted:** floxuridine

**KEY WORDS**
derivatization; serum; pharmacokinetics

**REFERENCE**
Yamaguchi,M.; Nakamura,M.; Kuroda,N.; Ohkura,Y. Determination of 5-fluorouracil and 5-fluoro-2'-deoxyuridine in human serum by high-performance liquid chromatography with fluorescence detection, *Anal.Sci.*, **1987**, *3*, 75–79.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Add 250 μL serum to a 20 × 7 DEAE-Cellulofine AM anion-exchange column (Seikagaku Tokyo), elute with 3.5 mL 1 mM HCl, discard the first 0.5 mL eluate, collect the next 3 mL eluate. Evaporate the eluate to 0.5 mL under reduced pressure, add 15 mL ethyl acetate, shake, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 800 μL anhydrous acetone, add 100 μL 750 μg/mL 4-bromomethyl-6,7-dimethoxycoumarin in acetone, add 100 μL 250 μg/mL 18-crown-6 in acetone, add 1.5 mg anhydrous potassium carbonate, heat at 70° for 15 min (protect from atmospheric moisture with a calcium chloride drying tube), cool, inject an aliquot

**HPLC VARIABLES**
**Column:** 200 × 4 5 μm Nucleosil 5 C18
**Mobile phase:** MeOH:water 60:40
**Flow rate:** 0.8
**Detector:** F ex 340 em 420

**CHROMATOGRAM**
**Retention time:** 10
**Limit of quantitation:** 60 ng/mL

**OTHER SUBSTANCES**
**Extracted:** floxuridine, ftorafur

**KEY WORDS**
derivatization; serum; protect from light

**REFERENCE**
Yoshida,S.; Adachi,T.; Hirose,S. 4-Bromomethyl-6,7-dimethoxycoumarin as a fluorescence reagent for precolumn derivatization of 5-fluorouracil compounds in high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 156–162.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Condition a 1 mL LC-SCX Supelclean strong cation-exchange SPE cartridge (Supelco) with 2 mL MeOH, 1 mL 100 mM copper(II) sulfate solution, and 3 mL 50 mM pH 7 phosphate buffer, do not allow to dry. 300 μL Serum + 5-bromouracil, add to the SPE cartridge, wash with 2 mL 50 mM pH 7 phosphate buffer, wash with 2 mL MeOH, elute with 700 μL 1.7 M ammonia solution, add 70 μL glacial acetic acid to the eluate, mix thoroughly, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 20 × 4.6 5 μm Supelguard LC-18-S (Supelco)
**Column:** 250 × 4.6 5 μm Supelcosil LC-18-S ODS

**Mobile phase:** Gradient. A was MeOH:50 mM pH 6.5 phosphate buffer 60:40. B was 50 mM pH 6.5 phosphate buffer.
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 269

**CHROMATOGRAM**
**Retention time:** 6.5
**Internal standard:** 5-bromouracil (12)
**Limit of detection:** 200 ng/mL

**OTHER SUBSTANCES**
**Extracted:** doxifluridine, floxuridine, 5-fluorouridine monophosphate, metabolites

**KEY WORDS**
serum; SPE

**REFERENCE**
Guerrieri,A.; Palmisano,F.; Zambonin,P.G.; De Lena,M.; Lorusso,V. Solid-phase extraction of fluoropyrimidine derivatives on a copper-modified strong cation exchanger: determination of doxifluridine, 5-fluorouracil and its main metabolites in serum by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *617*, 71–77.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1 mL Plasma + 100 μL 100 μg/mL niacinamide, add 2 mL MeCN dropwise, centrifuge at 3000 g for 30 min. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 1 mL saturated ammonium sulfate, add 5 mL diethyl ether:isopropanol 80:20, shake for 15 min, centrifuge at 3000 g for 15 min, repeat the extraction. Combine the organic phases and evaporate them to dryness, reconstitute the residue in 1 mL mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Guard column:** 5 μm LiChrosorb RP-18
**Column:** 250 × 4 5 μm LiChrosorb RP-18
**Mobile phase:** pH 7.0 phosphate buffer (μ = 0.05)
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 265

**CHROMATOGRAM**
**Retention time:** 5
**Internal standard:** niacinamide
**Limit of quantitation:** 690 ng/mL

**KEY WORDS**
plasma; pharmacokinetics

**REFERENCE**
Vandenbosch,C.; van Belle,S.; de Smet,M.; Taton,G.; Bruynseels,V.; Vandenhoven,G.; Massart,D.L. Determination of leucovorin and 5-fluorouracil in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 77–85.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 100 μL Plasma + 100 μL 20 μg/mL 5-bromouracil + 8 mL ethyl acetate, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 6 Shimpack CLS-ODS (Shimadzu)

**Mobile phase:** 0.5 mM phosphoric acid
**Column temperature:** 40
**Flow rate:** 1.5
**Injection volume:** 20
**Detector:** UV 280

**CHROMATOGRAM**
**Internal standard:** 5-bromouracil

**KEY WORDS**
plasma; rat

**REFERENCE**
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 100 μL Plasma + 50 μL 15 μg/mL 5-bromouracil + 1 mL 0.365% HCl + 8 mL ethyl acetate, shake for 10 min, centrifuge at 1006 g for 10 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 6 Shimpack CLS-ODS
**Mobile phase:** Water:phosphoric acid 100:0.1
**Column temperature:** 40
**Flow rate:** 1.7
**Injection volume:** 20
**Detector:** UV 270

**CHROMATOGRAM**
**Internal standard:** 5-bromouracil

**KEY WORDS**
plasma; rat; pharmacokinetics

**REFERENCE**
Umejima,H.; Kikuchi,A.; Kim,N.-S.; Uchida,T.; Goto,S. Preparation and evaluation of Eudragit gels. VIII. Rectal absorption of 5-fluorouracil from Eudispert hv gels in rats, *J.Pharm.Sci.*, **1995**, *84*, 199–202.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 200 μL Plasma + 50 μL 50 μg/mL stavudine + 1 mL ice-cold MeCN, mix vigorously for 30 s, centrifuge at 9000 g for 7 min. Remove the supernatant and add it to excess crystalline magnesium sulfate, mix for 2 min, centrifuge at 9000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 15-150 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 25 × 2.3 PRP-1 (Hamilton)
**Column:** 250 × 4.1 10 μm PRP-1 (Hamilton)
**Mobile phase:** MeCN:5 mM pH 11.1 tetrabutylammonium hydroxide 16:84
**Flow rate:** 1.5
**Injection volume:** 15-150
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 4.6
**Internal standard:** stavudine (5.5)
**Limit of quantitation:** 100 ng/mL

### OTHER SUBSTANCES
**Extracted:** 4-deoxy-5-fluorouracil (UV 313), tegafur

### KEY WORDS
plasma; rat; pharmacokinetics

### REFERENCE
Jarugula,V.R.; Boudinot,F.D. High-performance liquid chromatographic determination of 5-fluorouracil and its prodrugs, tegafur and 4-deoxy-5-fluorouracil, in rat plasma, *J.Chromatogr.B*, **1996**, *677*, 199–203.

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### SAMPLE
**Matrix:** blood, peritoneal fluid
**Sample preparation:** Plasma. 1 mL Plasma + 10 μL 100 μM bromouridine + 70 μL perchloric acid, mix thoroughly, let stand at 4° for at least 12 h, centrifuge for 5 min. Remove the supernatant and adjust the pH to 7 with 5 M KOH, let stand on ice for 2 h, inject a 20 μL aliquot. Peritoneal fluid. 1 mL Peritoneal fluid + 10 μL 100 μM bromouridine, dilute 1:100 with water, inject a 20 μL aliquot.

### HPLC VARIABLES
**Guard column:** 12.5 × 4.6 5 μm Zorbax RX
**Column:** 250 × 4.6 5 μm Zorbax RX
**Mobile phase:** Gradient. A was 25 mL pH 2.5 ammonium phosphate. B was MeCN:25 mM pH 7.5 ammonium phosphate 7:93. A:B 100:0 for 5 min, to 0:100 over 10 min, maintain at 0:100 for 10 min, return to initial conditions over 1 min, re-equilibrate for 20 min.
**Column temperature:** 20
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 270

### CHROMATOGRAM
**Retention time:** 6-7
**Internal standard:** bromouridine (18)
**Limit of detection:** 2.5 nM

### OTHER SUBSTANCES
**Extracted:** floxuridine

### KEY WORDS
plasma; pharmacokinetics

### REFERENCE
Smith-Rogers,J.A.; Tong,W.P.; Duafala,M.E.; Markman,M.; Bertino,J.R. High-performance liquid chromatographic method for the simultaneous measurement of floxuridine and fluorouracil in human body fluids, *J.Chromatogr.*, **1991**, *566*, 147–154.

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### SAMPLE
**Matrix:** blood, tissue
**Sample preparation:** Add 100 μL 10% perchloric acid and 20 μL 200 μg/mL IS to 100 μL serum or homogenized tissue. Shake for 2 min and centrifuge at 2000 g for 10 min. Filter (45 μm) supernatant, inject a 10 μL aliquot of the filtrate.

### HPLC VARIABLES
**Guard column:** 45 × 4.6 5 μm ODS Hypersil (VDS Optilab)
**Column:** 250 × 4.6 5 μm ODS Hypersil (VDS Optilab)
**Mobile phase:** MeOH:99% acetic acid:water 3:0.5:96.95
**Column temperature:** 30
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 4.94
**Internal standard:** 5-bromouracil (10.34)
**Limit of quantitation:** 100 ng/mL (serum); 300-500 ng/mL (tissue)

**OTHER SUBSTANCES**
**Extracted:** metabolites, floxuridine

**KEY WORDS**
serum; rat; liver; tumor; kidney; spleen; peritoneum; gastric mucosa; lung; heart; pancreas

**REFERENCE**
Jung,M.; Berger,G.; Pohlen,U.; Päuser,S.; Reszka,R.; Buhr,H.J. Simultaneous determination of 5-fluorouracil and its active metabolites in serum and tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 193–202.

**SAMPLE**
**Matrix:** blood, tissue
**Sample preparation:** 0.5 g Tissue or 1 mL plasma + 5 μL 1 M sulfuric acid (lung and heart only) + 2 μL 1 M sulfuric acid (plasma only) + 500 μL 200 mg/mL sodium sulfate (liver and kidney only) + 50 μL 1 M pH 6 sodium acetate (liver only) + 50 μL 1 M pH 5 sodium acetate (kidney only) + 100 μL 2% trichloroacetic acid (lung and heart only) + 15 mL n-propanol:ether (liver 16:84, kidney 20:80, lung 88:12, heart 40:60, plasma 88:12), sonicate for 30 s, shake for 15 min, centrifuge for 15 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 50 mM ammonium phosphate (liver pH 11, kidney pH 3, lung pH 2.5, heart pH 5, plasma pH 2.5), inject a 20 μL aliquot. (From J. Liq.Chromatogr. 1994, 17, 1621.)

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Spherisorb 5 ODS 2
**Mobile phase:** MeCN:50 mM phosphate buffer 0.5:99.5 (Liver pH 3, kidney pH 6, lung pH 5, heart pH 5, plasma pH 2.5)
**Column temperature:** 10 (kidney), 35 (lung), 20 (heart), 25 (plasma), 15 (liver)
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 200

**CHROMATOGRAM**
**Retention time:** 5 (plasma), 7 (liver), 7.5 (kidney), 8 (lung), 9 (heart)
**Internal standard:** flucytosine (for plasma) (4), 4-chlorouracil (for tissue) (18 (liver), 11 (kidney), 17 (lung), 19 (heart))
**Limit of quantitation:** 300 ng/g plasma, 800 ng/g (heart), 100 ng/g (lung), 240 ng/g (kidney), 570 ng/g (liver)

**OTHER SUBSTANCES**
**Extracted:** metabolites, floxuridine

**KEY WORDS**
plasma; rabbit; liver; kidney; lung; heart; pharmacokinetics

**REFERENCE**
Del Nozal,M.J.; Bernal,J.L.; Pampliega,A.; Marinero,P.; Pozuelo,M. Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 397–405.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject

a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 204

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**CHROMATOGRAM**
**Retention time:** 3.433

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**KEY WORDS**
whole blood

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**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

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**SAMPLE**
**Matrix:** bulk
**Sample preparation:** Dissolve 1-10 ng 5-fluorouracil in 100 μL DMSO, add 400 μL 2.5 mg/mL 4-bromomethyl-7-methoxycoumarin in DMSO, add 5 mg potassium carbonate, shake at room temperature for 15 min, add 500 μL water, centrifuge at 15600 g for 5 min, inject a 100 μL aliquot of the supernatant onto column A and elute to waste with mobile phase A, after 9.5 min divert the effluent containing the derivatized 5-fluorouracil onto column B, after another 2 min elute column B with mobile phase B, monitor the effluent from column B.

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**HPLC VARIABLES**
**Column:** A 150 × 4.6 5 μm CPS-Hypersil cyanopropyl; B 150 × 4.6 5 μm ODS-Hypersil
**Mobile phase:** A MeOH:water 50:50; B MeOH:water 60:40
**Column temperature:** 35 (column A only)
**Flow rate:** 1
**Injection volume:** 100
**Detector:** F ex 325 em 395

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**CHROMATOGRAM**
**Retention time:** 17
**Limit of detection:** 5 pg

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**KEY WORDS**
derivatization; column-switching; heart-cut

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**REFERENCE**
Kindberg,C.G.; Slavik,M.; Riley,C.M.; Stobaugh,J.F. High-performance liquid chromatography of 5-fluorouracil after derivatization with 4-bromomethyl-7-methoxycoumarin. Characterization of the derivative and the use of column switching for the improvement of resolution and the enhancement of sensitivity, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 459–469.

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**SAMPLE**
**Matrix:** formulations

**Sample preparation:** Condition a 500 mg SCX Tech-Elut strong cation exchange SPE cartridge (HPLC Technology) with 6 mL MeOH and five 2 mL portions of buffer. Tablets. Powder tablets, weigh out amount equivalent to 500 mg flucytosine, add 50 mL water, stir for 15 min, filter. 1 mL Filtrate + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, add 2 mL to the SPE cartridge, wash with 1 mL buffer, collect all the eluates, inject an aliquot. Injections. Dilute with water to a drug concentration of 10 mg/mL. 1 mL Sample + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, add 2 mL to the SPE cartridge, wash with 1 mL buffer, collect all the eluates, inject an aliquot. (Buffer was 70 mM $KH_2PO_4$ adjusted to pH 3.0 with HCl.) (To measure flucytosine as well as fluorouracil omit the SPE step.)

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Spherisorb CN
**Mobile phase:** 9 mM Sodium heptanesulfonate adjusted to pH 2.8 with phosphoric acid
**Flow rate:** 0.8
**Injection volume:** 20
**Detector:** UV 266

**CHROMATOGRAM**
**Retention time:** 4.0
**Internal standard:** thymine (4.85)

**OTHER SUBSTANCES**
**Simultaneous:** flucytosine (when SPE is omitted)

**KEY WORDS**
tablets; injections; SPE

**REFERENCE**
Cavrini,V.; Bonazzi,D.; Di Pietra,A.M. Analysis of flucytosine dosage forms by derivative UV spectroscopy and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 401–407.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute formulation 1:500 with water, inject a 50 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 5 × 4 35-60 μm Perisorb RP18
**Column:** 250 × 4 10 μm LiChrosorb RP18
**Mobile phase:** MeOH:300 mM sodium acetate 2:98
**Injection volume:** 50
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 4.7

**OTHER SUBSTANCES**
**Simultaneous:** floxuridine

**KEY WORDS**
injections; water

**REFERENCE**
Sadjak,A.; Wintersteiger,R. Compatibility of morphine, baclofen, floxuridine and fluorouracil in an implantable medication pump, *Arzneimittelforschung*, **1995**, *45*, 93–98.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute 1 mL formulation to 10 mL with mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.6 μBondapak phenyl

**Mobile phase:** MeCN:MeOH:0.01% acetic acid:0.005 N sulfonic acid 20:15:40:25
**Flow rate:** 1
**Detector:** UV 230

**CHROMATOGRAM**
**Retention time:** 3.5

**OTHER SUBSTANCES**
**Simultaneous:** metoclopramide

**KEY WORDS**
injections; 5% dextrose; stability-indicating

**REFERENCE**
Wang,D.-P.; Chang,L.-C.; Lee,D.K.T.; Wong,C.-Y. Stability of fluorouracil-metoclopramide hydrochloride admixture, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 98–99.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute with mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm C18
**Mobile phase:** MeCN:100 mM $NaH_2PO_4$ 20:80 adjusted to pH 4.2 with phosphoric acid
**Flow rate:** 2.5
**Injection volume:** 20
**Detector:** UV 300

**CHROMATOGRAM**
**Retention time:** 1.58

**OTHER SUBSTANCES**
**Simultaneous:** cytarabine, granisetron

**KEY WORDS**
stability-indicating; injections; saline

**REFERENCE**
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Guard column:** 10 μm precolumn (Beckman Instruments Inc.)
**Column:** 150 × 4.6 5 μm C18 Altex Ultrasphere ODS
**Mobile phase:** Isocratic. MeOH:buffer 15:85 containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. Gradient. A was MeOH containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. B was buffer containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. A:B 0:100 for 2 min, to 30:70 over 1 min, maintain at 30:70. (Buffer was 50 mM phosphoric acid containing 50 mM $KH_2PO_4$, pH 2.5.)
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 254; UV 285

**CHROMATOGRAM**
**Retention time:** 2.52 (isocratic), 4.36 (gradient)

**Internal standard:** 5-methylcytosine (5.66 (isocratic), 12.62 (gradient))
**Limit of quantitation:** 12.5 μM

**OTHER SUBSTANCES**
**Simultaneous:** barbituric acid, cytosine, flucytosine, hydroxycytosine, uracil, urea

**REFERENCE**
Biondi,L.; Nairn,J.G. High performance liquid chromatographic assay for 5-fluorouracil and 5-fluorocytosine, *J.Liq.Chromatogr.*, **1985**, *8*, 1881–1892.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Dilute a 50 mg/mL sample 1:100 with mobile phase and inject an aliquot.

**HPLC VARIABLES**
**Column:** Bakerbond C18
**Mobile phase:** 5 mM pH 7.8 $K_2HPO_4$
**Flow rate:** 0.5
**Detector:** UV 214

**CHROMATOGRAM**
**Retention time:** 6.6

**OTHER SUBSTANCES**
**Simultaneous:** degradation products

**KEY WORDS**
stability-indicating

**REFERENCE**
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject an aliquot of an aqueous solution.

**HPLC VARIABLES**
**Column:** 100 × 8 Resolve C18
**Mobile phase:** MeCN:50 mM ammonium dihydrogen phosphate 4:96, pH adjusted to 3.5 with $H_3PO_4$
**Flow rate:** 1.7
**Detector:** UV 268

**CHROMATOGRAM**
**Retention time:** 2.8

**OTHER SUBSTANCES**
**Simultaneous:** fluorouridine

**REFERENCE**
Dorta,M.J.; Munguia,O.; Fariña,J.B.; Martin,V.S.; Llabrés,M. Stability indicating high performance liquid chromatography methods for 5-fluorouridine in aqueous solution, *Arzneimittelforschung*, **1997**, *47*, 1388–1392.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
**Column temperature:** 30
**Flow rate:** 2
**Detector:** UV 210

## OTHER SUBSTANCES

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 150 mm long 5 μm Microsorb-MV C18
**Mobile phase:** MeCN:pH 4 sodium acetate buffer 2:98
**Flow rate:** 1.5

**Detector:** UV 270

### REFERENCE

Phillips,C.A.; Michniak,B.B. Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers, *J.Pharm.Sci.*, **1995**, *84*, 1427–1433.

### SAMPLE

**Matrix:** tissue

**Sample preparation:** Homogenize (Ultra-Turrax) 1 g tissue with 0.05-1 μg IS and 15 mL ice-cold MeCN, rinse homogenizer with 10 mL ice-cold MeCN, centrifuge at 6000 rpm for 15 min, remove the supernatant, wash the pellet with 5 mL MeCN, centrifuge for 5 min. Combine the supernatants, remove a 5 mL aliquot, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL n-hexane:ethyl acetate:water 40:80:1, chromatograph on a 125 × 4 5 μm Spherisorb Si column (A) and a 200 × 4.6 5 μm Spherisorb Si column (B) with n-hexane:ethyl acetate:water 40:80:1 at 1 mL/min using column-switching. Initially elute the columns in series, after 3 min (after 5-fluorouracil has eluted from column A to column B) elute only column B, monitor the effluent from column B at 266 nm, collect the appropriate fraction. (Backflush column A to waste for 6 min before the next injection.) Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 10 μL acetone, add 1 mg freshly-powdered potassium carbonate, add 5 μL 200 μg/mL 18-crown-6 in acetone, add 20 μL 750 μg/mL 4-bromomethyl-7-methoxycoumarin in acetone, heat at 70° for 25 min, inject a 1 μL aliquot.

### HPLC VARIABLES

**Guard column:** 20 × 1.6 3 μm Nukleosil-120 (MZ Analysentechnik)
**Column:** 125 × 1.6 3 μm Nukleosil-120 (MZ Analysentechnik)
**Mobile phase:** MeCN:MeOH:water 30:15:50
**Flow rate:** 0.06
**Injection volume:** 1
**Detector:** F ex 305 em 407

### CHROMATOGRAM

**Retention time:** 27
**Internal standard:** 5-chlorouracil (31)
**Limit of detection:** 3 ng/g
**Limit of quantitation:** 30 ng/g

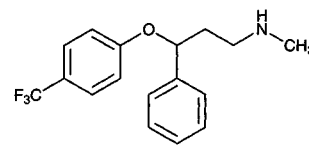
### KEY WORDS

derivatization; microbore; human; pig; liver

### REFERENCE

Jochheim,C.; Janning,P.; Marggraf,U.; Löffler,T.M.; Hasse,F.; Linscheid,M. A procedure for the determination of 5-fluorouracil in tissue using microbore HPLC and fluorescence detection, *Anal.Biochem.*, **1994**, *217*, 285–291.

# Fluoxetine




**Molecular formula:** $C_{17}H_{18}F_3NO$
**Molecular weight:** 309.33
**CAS Registry No.:** 54910-89-3, 59333-67-4 (HCl)
**Merck Index:** 4222
**Lednicer No.:** 3 32

### SAMPLE

**Matrix:** blood

**Sample preparation:** Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for

10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
**Mobile phase:** MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
**Flow rate:** 1.2
**Injection volume:** 100
**Detector:** UV 226, UV 254, UV 400

**CHROMATOGRAM**
**Retention time:** 15.50
**Internal standard:** clovoxamine (6.5)
**Limit of quantitation:** 5 ng/mL (UV 226, UV 400); 7 ng/mL (UV 254)

**OTHER SUBSTANCES**
**Extracted:** metabolites, amitriptyline, clomipramine, desipramine, imipramine, maprotiline, nortriptyline
**Simultaneous:** amineptine, carbamazepine, chlordiazepoxide, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
**Noninterfering:** diazepam, valproic acid
**Interfering:** chlorpromazine, clonazepam

**KEY WORDS**
plasma

**REFERENCE**
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 1 mL plasma with 400 μL 50 μg/mL IS in MeOH, make up to 2 mL with MeOH, centrifuge at 1100 g for 10 min. Remove a 1 mL aliquot of the supernatant, add 2 mL 1 M NaOH and 2 mL diethyl ether, shake for 10 min, repeat the extraction. Combine the ether extracts and dry them over anhydrous sodium sulfate. Evaporate to dryness under a stream of nitrogen and reconstitute the residue in 1 mL MeOH. Inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 200 × 4 10 μm LiChrosorb RP-18
**Mobile phase:** MeCN:pH 2.7 phosphate buffer 90:10
**Flow rate:** 1.0
**Injection volume:** 20
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 2.53
**Internal standard:** chlorprothixene (5.20)
**Limit of detection:** 100 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** amitriptyline, azathioprine, diazepam, doxepin, fludarabine, imipramine, mercaptopurine,
**Interfering:** methadone, pentazocine, piritramide, tramadol

**KEY WORDS**
plasma

**REFERENCE**
Misztal,G.; Hopkala,H. Determination of fluoxetine in human plasma using reversed phase HPLC, *Pharmazie*, **1997**, *52*, 854–856.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Add 400 μL 330 mM NaOH to 2 mL plasma. Mix for 5 min, extract with 14 mL n-hexane:isoamyl alcohol 98.5:1.5 for 20 min. Centrifuge at 2500 g for 5 min and collect the organic layer. Adjust to pH 2 with 400 μL HCl, shake for 1 min, centrifuge at 1500 g for 10 min. Inject a 100 μL aliquot of the aqueous layer.

**HPLC VARIABLES**
**Guard column:** 15 × 4.6 5 μm LiChrospher RP-18
**Column:** 250 × 4.6 5 μm Spherisorb ODS-2
**Mobile phase:** MeCN:5 mM n-octylamine in water 40:60, adjusted to pH 6.4 with orthophosphoric acid (After use, wash the column with water for 15 min, MeCN:water 50:50 for 15 min, and MeCN for 5 min.)
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 230

**CHROMATOGRAM**
**Retention time:** 24.41
**Limit of detection:** 4.5 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites
**Simultaneous:** amisulpride, diazepam, flunitrazepam, fluvoxamine, haloperidol, imipramine, maprotiline, paroxetine, amitriptyline, clomipramine, mianserin

**KEY WORDS**
plasma

**REFERENCE**
Gennaro,M.C.; Abrigo,C.; Angelino,S.; Albert,U.; Bogetto,F.; Maina,G.; Prolo,P.; Ravizza,L. Determination of fluoxetine and norfluoxetine in human plasma by ion-interaction RP-HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3017–3028.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 100 μL Serum + 20 μL 5 μg/mL IS in MeOH + 100 μL 5 M NaOH, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it under a gentle stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Guard column:** RP C18 (Brownlee)
**Column:** 150 × 4.6 5 μm Microsorb MV
**Mobile phase:** MeCN:water 55:45 containing 10 mM triethylamine, adjusted to pH 4.8 with 85% phosphoric acid
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 226

**CHROMATOGRAM**
**Retention time:** 4.7
**Internal standard:** clomipramine (7.4)
**Limit of quantitation:** 25 ng/mL

### OTHER SUBSTANCES
**Extracted:** norfluoxetine

### KEY WORDS
mouse; serum; pharmacokinetics

### REFERENCE
Holladay,J.W.; Dewey,M.J; Yoo,S.D. Quantification of fluoxetine and norfluoxetine serum levels by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1997**, *704*, 259–263.

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### SAMPLE
**Matrix:** blood, tissue
**Sample preparation:** 100 μL Serum or 200 μL brain homogenate + 20 μL 5.0 μg/mL clomipramine in MeOH + 100 μL 5.0 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 5 min. Evaporate organic layer under a gentle stream of nitrogen at 20°. Reconstitute residue with 50 μL mobile phase, inject a 20 μL aliquot.

### HPLC VARIABLES
**Column:** Microsorb MV C18 (Rainin, Woburn, USA)
**Mobile phase:** MeCN:water 55:45 containing 10 mM triethylamine, pH adjusted to 4.8 with 85% phosphoric acid
**Flow rate:** 1.0
**Injection volume:** 20
**Detector:** UV 226

### CHROMATOGRAM
**Internal standard:** clomipramine
**Limit of quantitation:** 25 ng/mL

### OTHER SUBSTANCES
**Extracted:** metabolites

### KEY WORDS
serum; brain; mouse; pharmacokinetics

### REFERENCE
Holladay,J.W.; Dewey,M.J.; Yoo,S.D. Pharmacokinetics and antidepressant activity of fluoxetine in transgenic mice with elevated serum α-1-acid glycoprotein levels, *Drug Metab.Dispos.*, **1998**, *26*, 20–24.

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### SAMPLE
**Matrix:** blood, tissue
**Sample preparation:** 100 μL Serum or weighed homogenized brain + 1 mL 600 mM pH 9.8 sodium carbonate-sodium bicarbonate buffer containing 100 ng/mL IS. Add 7 mL ethyl acetate: n-heptane 20:80, mix vigorously for 1.5 min, centrifuge at 3000 g for 10 min. Mix the organic layer with 200 μL 25 mM potassium dihydrogen phosphate adjusted to pH 2.3 with 85% phosphoric acid. Mix for 1 min, centrifuge at 3000 g for 10 min, discard the organic layer, inject a 50 μL aliquot of the aqueous phase.

### HPLC VARIABLES
**Guard column:** 4 × 4 5 μm C8 endcapped (Merck, Germany)
**Column:** 125 × 4 4 μm C8 endcapped (Merck, Germany)
**Mobile phase:** MeCN:buffer 42:58 (Buffer was water containing 100 μL/L perchloric acid and 1.5 g/L tetramethylammonium perchlorate.)
**Flow rate:** 1.2
**Injection volume:** 50
**Detector:** UV 227

### CHROMATOGRAM
**Retention time:** 12.7
**Internal standard:** protriptyline (8.1)

**Limit of detection:** 5 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites
**Simultaneous:** acetopromazine, aceprometazine, amitriptyline, bromazepam, chlorpromazine, ciamemazine, clomipramine, clorazepate, demethyldomipramine, diazepam, flunitrazepam, imipramine, nitrazepam, nordiazepam, nortriptyline

**KEY WORDS**
serum; brain; mouse; human; pharmacokinetics

**REFERENCE**
Alvarez,J.-C.; Bothua,D.; Collignon,I.; Advenier,C.; Spreux-Varoquaux,O. Determination of fluoxetine and its metabolite norfluoxetine in serum and brain areas using high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1998**, *707*, 175–180.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

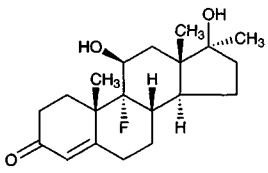
**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200.5

**CHROMATOGRAM**
**Retention time:** 16.185

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

# Fluoxymesterone




**Molecular formula:** $C_{20}H_{29}FO_3$
**Molecular weight:** 336.45
**CAS Registry No.:** 76-43-7
**Merck Index:** 4223
**Lednicer No.:** 1 175